Supplemental Figure Legends:

Supplementary Fig. S1. WT recombinant NPL shows ~3-fold reduced activity against Neu5Gc as compared with Neu5Ac substrate. Specific NPL activity (nmol/h/mg of protein) of recombinant WT human NPL expressed in HEK was measured against different concentrations of Neu5Gc and Neu5Ac substrates as described in Materials and methods. Enzymatic activity of the WT enzyme against KDN or of NPL p.N45D and p.R63C mutants against Neu5Gc or KDN substrates was below detection level. Data show means and SEM of three independent experiments and their analysis by non-linear regression (Michaelis-Menten equation curve fit) using GraphPad software.

Supplementary Fig. S2. Expression pattern of *npl* during development in zebrafish. (A-F) Whole mount *in situ* hybridization of *npl* on wild type embryos at 24 and 48 hpf, and 3 and 4 dpf using antisense, or **(G-L)** sense probes. **(A- C**, and **E**, and **G-I,** and **K)** Lateral views. **(D** and **F**, and **J** and **L)** Dorsal views. Arrows in **(D** and **F)** point to the fin bud. Arrow in **(E)** points to the gut. (Scale bar: 125μm.)

Supplementary Fig. S3. NPL activity is significantly reduced in *npl* atg MO and restored in in *npl* atg MO treated with WT npl mRNA. NPL activity was measured with Neu5Ac as a substrate as described in Materials and methods. Data show means, SEM and individual values of 3 independent experiments each performed with 25 pooled fish embryos. ** and *** significantly different (P<0.01 and 0.001, resp[ectively) in one-way ANOVA test.

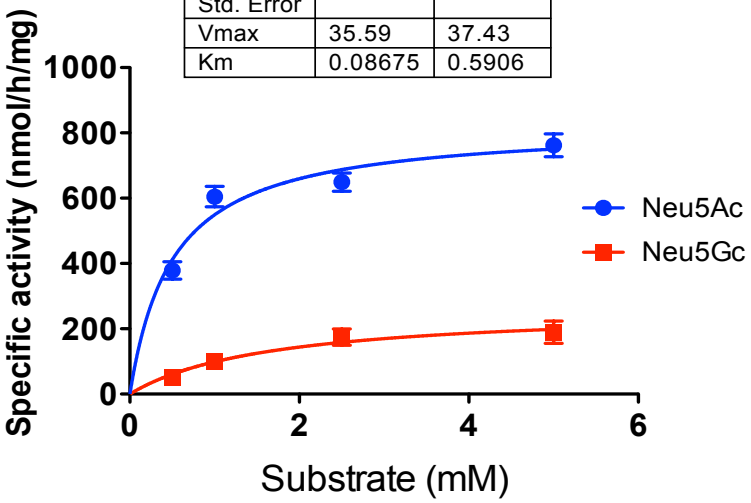
Supplementary Fig. S4. npl morphants have abnormal somite morphology as revealed by *in situ* hybridization of *myod*. Whole mount *in situ* hybridization against *myod* on embryos injected with Cont MO **(A, C)** or *npl*-atg MO **(B, D)** at 24 and 48 hpf. **(A-D)** Close ups of boxed areas showing somites (arrows) and **(C,D**) close ups of the head showing loss of expression of *myod* in ocular and facial muscles (scale bar: 125μm).

Supplementary Fig. S5. Injection of a *npl* splicing morpholino affects muscle development in zebrafish embryos. (A) Diagram of the zebrafish *npl* gene indicating the position of the splicing morpholino targeting the exon 4/intron 4 boundary (*npl*-sp MO), and the primers used to detect the aberrantly spliced transcript (blue arrows). **(B)** Lateral views of zebrafish embryos at 48 hpf that were injected with a Cont MO, or *npl* sp MO. Morphants present pericardial edema and severe somite disorganization (boxes, scale bar: 750

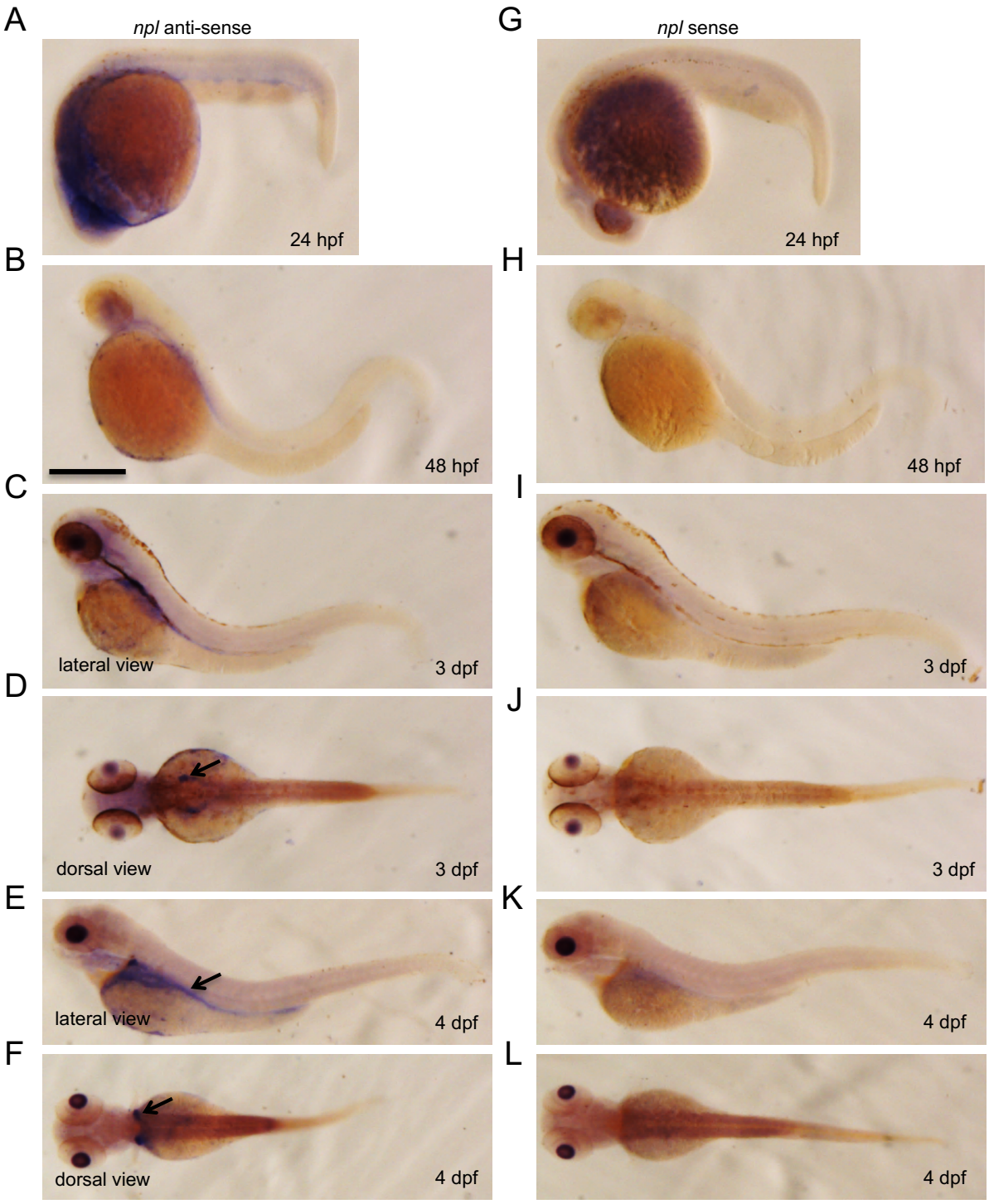
μm). **(B)** Confocal images of muscle fibers in somites (arrows) immunostained for phalloidin on 48 hpf embryos (scale bar: 50 μm). **(C)** RT-PCR for *npl* using RNA from embryos that were injected with Cont MO or *npl*-sp MO. **(D)** Confocal images of muscle fibers in somites (arrows) immunostained for phalloidin on 48 hpf embryos that were injected with Cont MO or *npl* sp ex4 MO, and embryos injected with *npl* sp ex4 MO that were treated with 800 μM ManNAc (scale bar: 50 μm).

Supplementary Figure S1

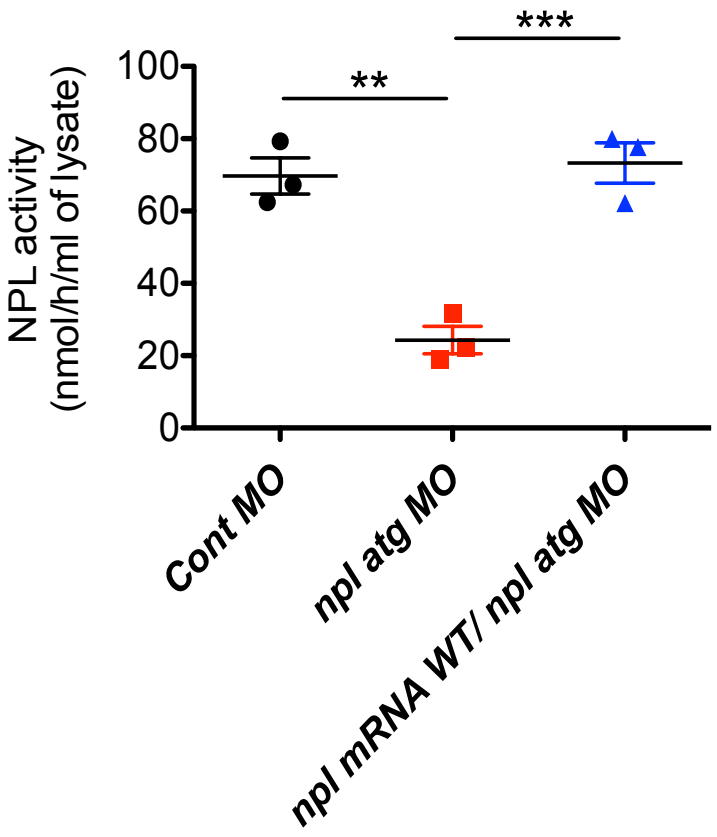
	Neu5Ac	Neu5Gc
Vmax	826.2	265.1
Km	0.5048	1.682
Std. Error		
Vmax	35.59	37.43
Km	0.08675	0.5906



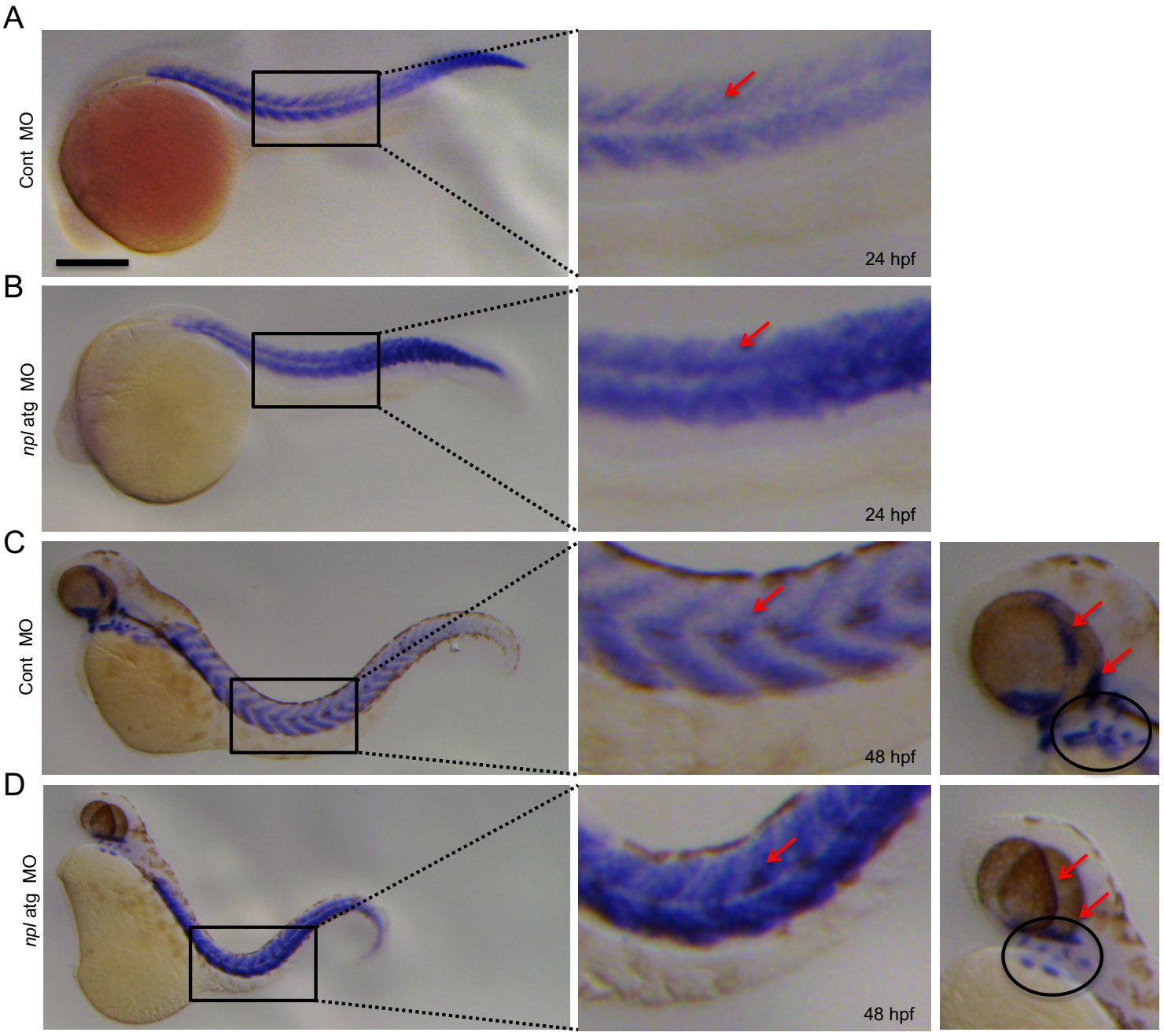
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5